A regulatory role for suppressor of cytokine signaling-1 in T_h polarization in vivo

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Keywords: JAK, STAT, STAT-induced STAT inhibitor, suppressor of cytokine signaling-1, T_h1, T_h2

Abstract

Suppressor of cytokine signaling (SOCS)-1 is an inhibitory molecule for JAK, and its deficiency in mice leads to lymphocyte-dependent multi-organ disease and perinatal death. Crossing of SOCS-1−/− mice on an IFN-γ−/−, STAT1−/− and STAT6−/− background revealed that the fatal disease of SOCS-1−/− mice is also dependent on IFN-γ/STAT1 and IL-4/STAT6 signaling pathways. Since IFN-γ and IL-4 are representative T_h1 and T_h2 cytokines respectively, here we investigated the role of SOCS-1 in T_h differentiation. Freshly isolated SOCS-1−/− CD4+ T cells stimulated with anti-CD3 rapidly produced larger amounts of IFN-γ and IL-4 than control cells, suggesting that these mutant T cells had already differentiated into T_h1 and T_h2 cells in vivo. In addition, SOCS-1+/− CD4+ T cells cultured in vitro produced significantly larger amounts of IFN-γ and IL-4 than SOCS-1+/+ cells. Similarly, SOCS-1+/− CD4+ T cells produced more IFN-γ and IL-4 than SOCS-1+/+ cells after infection with Listeria monocytogenes and Nippostrongylus brasiliensis respectively. Since IL-12-induced STAT4 and IL-4-induced STAT6 activation is sustained in SOCS-1−/− T cells, the enhanced T_h functions in SOCS-1−/− and SOCS-1+/− mice appear to be due to the enhanced effects of these cytokines. These results suggest that SOCS-1 plays a regulatory role in both T_h1 and T_h2 polarizations.

Introduction

CD4+ T cells play a critical role in immune responses following microbial infection (1–3). After stimulation with pathogens, these cells differentiate into two subsets known as T_h1 and T_h2 cells. These T_h1 and T_h2 cells are characterized by their profiles of cytokine production such as IFN-γ and IL-4 respectively. Commitment to T_h1 or T_h2 cells is essential for eradication of various pathogens and also plays a role in the induction of various diseases, including autoimmune diseases and allergy. Despite considerable efforts, the signals that induce the commitment of naive CD4+ T cells into T_h1 or T_h2 cells are still to be elucidated. However, many studies suggest that multiple factors influence T_h commitment at various stages.

Cytokines are profoundly involved in the process of CD4+ T cell differentiation (2,3). In particular, signals of IL-12 and IL-4 are relevant to T_h1 and T_h2 differentiation respectively. Recent characterization of suppressor of cytokine signaling (SOCS) / STAT-induced STAT inhibitor/cytokine-inducible SH2-containing protein (CIS) family proteins has revealed that cytokines are, at least in part, negatively regulated by the members of this family (4–6). Among these proteins, SOCS-1 appears to have the most broad inhibitory effect on cytokines including IL-4, IFN-γ and possibly IL-12 in vitro, since SOCS-1 interacts with and inhibits all four members of JAK that play essential roles in the signaling by various cytokines.

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Transmitting editor: L. H. Glimcher
Received 29 March 2002, accepted 10 August 2002

International Immunology, Vol. 14, No. 11, pp. 1343–1350 ã 2002 The Japanese Society for Immunology

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A regulatory role for SOCS-1 in Th polarization in vivo

Generation of SOCS-1−/− mice revealed the critical roles of SOCS-1 in vivo (7,8). Although SOCS-1−/− mice appear normal at birth, they exhibit growth retardation with aging and multi-organ inflammatory changes with mononuclear cell infiltration, and die before 3 weeks of age (7,8).

Intriguingly, T cells isolated from SOCS-1−/− mice are endogenously activated (9,10) and SOCS-1/−/− double knockout (DKO) mice which lack mature lymphocytes are rescued from this fatal disease (10). These SOCS-1−/− T cells appear to aberrantly produce IFN-γ in vivo, since significant elevation of serum IFN-γ is observed in SOCS-1−/− mice (10). In addition, IFN-γ is critically involved in the disease of SOCS-1−/− mice since SOCS-1−/−/− IFN-γ DKO mice are also free from early death (11). Recently, another study revealed a defect of SOCS-1/−/− CD4+ T cells in IL-6-mediated inhibition of Th1 death (11). These SOCS-1−/− T cells to the action of IFN-γ is observed in SOCS-1−/− mice (10). In

Moreover, however, we demonstrated that SOCS-1/−/−STAT6 DKO mice and SOCS-1−/−/−STAT1 DKO mice, which are defective in IL-4 and IFN-γ signaling respectively, survive much longer than SOCS-1−/− mice, and exhibit milder thymic and splenic atrophy with a remarkable reduction in numbers of activated T cells (9). This finding appears to suggest that SOCS-1−/− mice are injured by endogenous accumulation not only of IFN-γ, but also of IL-4.

In the present study, to precisely evaluate a role for SOCS-1 in the immune systems, we examined whether SOCS-1 levels influence Th1 and/or Th2 differentiation. Freshly isolated SOCS-1−/− CD4+ T cells and cultured SOCS1+/− CD4+ T cells produced larger amounts of both IL-4 and IFN-γ than control cells. In addition, SOCS-1−/− mice exhibited hyper-polarization into Th1 and Th2 phenotypes upon infection with Listeria monocytogenes and Nippostrongylus brasiliensis respectively.

Collectively, our findings suggest that SOCS-1 insufficiency leads to the enhanced Th1 and Th2 responses, but not selective polarization into Th1 cells.

Methods

Mice

The generation of SOCS-1−/−, SOCS-1/−/−STAT6 DKO and SOCS-1−/−/− mice was reported previously (8,9). All the SOCS-1−/− mice including DKO mice and their littermate controls were used at 2–3 weeks of age, while SOCS-1−/− mice and their littermate controls were used at 6–12 weeks of age. Mice were kept in specific pathogen-free facilities.

Cell preparation

Single-cell suspensions were obtained from thymuses and spleens as described previously (13). Where indicated, splenic CD4+ T cells were purified using anti-CD4–FITC (PharMingen, San Diego, CA), anti-FITC beads and a magnetic cell sorter (MACS; Miltenyi Biotec, Gladbach, Germany) according to the manufacturer’s instructions.

Flow cytometric analysis

Splenocytes were incubated with indicated antibodies and analyzed on a FACS Calibur (Becton Dickinson, San Jose, CA) using CellQuest software. The antibodies used were FITC-, phycoerythrin-, PerCP- and allophycocyanin-conjugated anti-CD4, -CD8, -B220 and -IgM (PharMingen).

Cell culture

Lymphocytes were cultured in RPMI 1640 supplemented with 10% FCS (JRH Biosciences, Lenexa, KS), 2-mercaptoethanol (Nacalai Tesque, Kyoto, Japan), penicillin G and streptomycin. Splenic CD4+ T cells were stimulated with plate-bound anti-CD3ε plus anti-CD28 (1 μg/ml each; PharMingen) and culture supernatants were harvested daily for ELISA. For in vitro Th1/Th2 polarization, CD4+ T cells were stimulated with plate-bound anti-CD3ε plus anti-CD28 (1 μg/ml) in the presence of murine IL-2 (10 ng/ml; PeproTech, London, UK). For Th1 differentiation, murine IL-12 (10 ng/ml; PharMingen) and anti-IL-4 (10 μg/ml; PharMingen) were added to the culture media, and for Th2 differentiation, murine IL-4 (10 ng/ml; PeproTech), anti-IL-12 and anti-IFN-γ (10 μg/ml each; PharMingen) were added. After 4 days, these cells were harvested, washed and re-stimulated with plate-bound anti-CD3 plus anti-CD28 (1 μg/ml). After 24 h, culture supernatants were obtained for ELISA.

Intracellular cytokine staining

Naive CD4+ T cells (CD4+CD62Lhi cells) were enriched from spleens. In brief, CD4+ T cells were obtained from spleen cells using SpinSep Murine CD4 T cell (Stemcell Technologies, Vancouver, Canada) according to the manufacturer’s instructions. These cells were then positively sorted with anti-CD62L beads and MACS systems (Miltenyi Biotec). Obtained cells were stimulated in vitro for 4 days in Th1 or Th2 conditions as described above and harvested 5 h after re-stimulation with plate-bound anti-CD3 (5 μg/ml) in the presence of Monensin (3 nM; Sigma, St Louis, MO) for the last 2.5 h. These cells were then treated with CyChrome-labeled anti-CD4 (PharMingen) and then stained with the indicated anti-cytokine antibodies (PharMingen) using the Cytotox/Cytoperm kit (PharMingen). Flow cytometric analysis was performed on a FACS Calibur. Results were shown for CD4+ live T cells determined by FSC and SSC profiles.

Western blot analysis

Thymocytes were stimulated with concanavalin A (2 μg/ml; Sigma) for 3 days, harvested, washed 3 times with PBS, counted and re-stimulated with murine IL-12 (20 ng/ml; PharMingen) or murine IL-4 (20 ng/ml; PeproTech) for 20 min. After vigorous washing and incubation in culture media for indicated minutes, cells were lysed with ice-cold buffer containing 1% Nonidet P-40, 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM Na3VO4 and 10 μM aprotinin (Sigma). Western blotting was performed as described previously (14). The antibodies used here were anti-phospho-STAT4 (Zymed, San Francisco, CA), anti-STAT4 (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phospho-STAT6 (Cell Signaling, Beverly, MA), anti-STAT6 (R & D Systems, Minneapolis, MN) and horseradish peroxidase-
conjugated anti-rabbit Ig (Amersham Pharmacia, Little Chalfont, UK).

Assays for cytokines
Cytokines (IL-2, IL-4 and IFN-γ) in culture supernatants were determined by ELISA kits (BioSource International, Camarillo, CA) according to the manufacturer’s instructions.

Infection with L. monocytogenes and N. braziliensis
L. monocytogenes 43251 purchased from ATCC (Manassas, VA) was cultured in Trypsicase Soy Broth (Becton Dickinson). Mice with various genotypes were infected with $5 \times 10^5$ L. monocytogenes through a tail vein or were inoculated s.c. with 400 N. braziliensis as shown previously (15). At day 14, splenic CD4 T cells were prepared using MACS (Miltenyi Biotec) following incubation with anti-CD4 beads (Miltenyi Biotec) and activated with plate-bound anti-CD3e (PharMingen) for 48 h. Concentrations of IFN-γ and IL-4 in each supernatant were determined by ELISA (Genzyme TECHNE, Boston, MA). In the case of N. braziliensis infection, serum was sampled at indicated time points for measurement IgE by ELISA (PharMingen).

Results
Improved phenotype of T cells in STAT1- or STAT6-depleted SOCS-1±/± mice
As we reported previously, both SOCS-1/STAT1 and SOCS-1/STAT6 DKO mice exhibited significant restoration from the hepatic disease caused by SOCS-1 deficiency and survived longer than mice lacking SOCS-1 alone (9). In addition, as shown in Fig. 1, the lymphocyte depletion observed in spleen of SOCS-1±/± mice was significantly improved both in SOCS-1/STAT1 and in SOCS-1/STAT6 DKO mice. These results indicate that IFN/STAT1 and IL-4/STAT6 signaling are both involved in the disease progression of SOCS-1±/± mice and affect the homeostasis of peripheral lymphocytes.

SOCS-1±/± CD4+ T cells spontaneously differentiate into T_h1 and T_h2 cells
Because IFN-γ and IL-4 are hallmark cytokines generated by T_h1 and T_h2 cells respectively, the above results suggest that CD4+ T cells of SOCS-1±/± mice may spontaneously differentiate into T_h1 and T_h2 cells in vivo. Therefore, we next stimulated splenic CD4+ T cells from SOCS-1±/± or SOCS-1±/± mice with plate-bound anti-CD3 for 24 h. CD4+ T cells prepared from SOCS-1±/± mice rapidly produced extremely...
large amounts of both IFN-γ and IL-4 compared with those from control mice (Fig. 1B). A similar result was obtained by flow cytometric analysis with intracellular staining of IFN-γ and IL-4 (data not shown). These results extend the previous findings that SOCS-1−/− T cells are spontaneously activated (9,10), and suggest that these CD4+ T cells have already differentiated into Th1 and Th2 cells in vivo. Thus, although the stimuli that drive SOCS-1−/− CD4+ T cells to differentiate into Th1/Th2 cells are currently unknown, it is likely that SOCS-1 deficiency leads to enhanced Th1/Th2 responses in vivo.

SOCS-1 levels affect production of both IL-4 and IFN-γ after stimulation with anti-CD3/anti-CD28

Next, we used SOCS-1+/− CD4+ T cells instead of SOCS-1−/− cells in vitro. This is because SOCS-1−/− mice are healthy and contain normal numbers of naive CD4+ T cells which we failed to obtain sufficiently from SOCS-1−/− mice due to accelerated apoptosis and spontaneous activation of SOCS-1−/− T cells (8). Furthermore, it was reported that inactivation of a single SOCS-1 allele has biological effects sufficient to restore the lactation defect in PRLR−/− mice (16). We first investigated the role of SOCS-1 in cytokine production by CD4+ T cells upon TCR engagement. SOCS-1+/− CD4+ T cells as well as SOCS-1−/− control cells were stimulated with plate-bound anti-CD3 plus anti-CD28 and culture supernatants were harvested daily to examine the secretion of cytokines. As shown in Fig. 2(A), similar amounts of IL-2 were produced both in SOCS-1+/− and SOCS-1−/− CD4+ T cells, suggesting that the lack of a single SOCS-1 allele does not affect signaling of CD3/CD28 pathways. However, as shown in Fig. 2(B), production of IFN-γ was enhanced in SOCS-1−/− mice. This result is consistent with those of previous studies demonstrating a role of SOCS-1 in the regulation of IFN-γ secretion in vivo (10) and in vitro (12). In addition, interestingly, production of IL-4 was also enhanced in SOCS-1+/− CD4+ T cells (Fig. 2C). These results indicate that SOCS-1−/− CD4+ T cells can be polarized more easily and/or rapidly than SOCS-1+/− CD4+ cells, and suggest a role for SOCS-1 in the negative regulation of both Th1 and Th2 responses.

SOCS-1−/− CD4+ T cells have enhanced potential to develop into Th1 and Th2 cells in vitro

We next incubated these cells under neutral, Th1- or Th2-polarizing culture conditions in vitro. As shown in Fig. 3(A), modestly elevated production of IFN-γ under Th1-polarizing conditions was observed in SOCS-1−/− CD4+ T cells. In addition, IL-4 production under Th2 polarization was also enhanced in SOCS-1−/− CD4+ T cells (Fig. 3B). It should be noted that IFN-γ production under Th2 conditions as well as IL-4 production under Th1 conditions were not enhanced in SOCS-1−/− cells. We also analyzed the status of Th polarization by another method. In this experiment, we used purified naive CD4+ T cells, cultured these cells under Th1-, Th2-polarizing conditions and analyzed them by intracellular cytokine staining. As shown in Fig. 3(C), enhanced Th1 polarization was observed again in SOCS-1−/− CD4+ T cells. Compared with the result shown in Fig. 2, the enhancement of IFN-γ/IL-4 production by SOCS-1−/− CD4+ T cells on these Th1/Th2-polarizing conditions was less significant. However, in repeated experiments, when SOCS-1+/− CD4+ T cells were compared to SOCS-1−/− cells, IL-4 as well as IL-10 production under Th2 conditions was always more marked than IFN-γ production under Th1 conditions. These results suggest again that SOCS-1 negatively regulates not only the process of Th1 differentiation, but also that of Th2 differentiation.

SOCS-1 levels influence the intensity of both Th1 and Th2 polarization in vivo

The impact of SOCS-1 insufficiency on Th1/Th2 cell differentiation was further confirmed by in vivo experiments. SOCS-1+/− and SOCS-1−/− mice were infected with L. monocytogenes, an intracellular facultative Gram-positive bacterium which induces Th1 responses in hosts. Fourteen days after infection, splenic CD4+ T cells were stimulated in vitro with immobilized anti-CD3 to evaluate the status of Th polarization. Consistent with the data obtained in vitro, CD4+ T cells from SOCS-1+/− mice produced larger amounts of IFN-γ than those from SOCS-1−/− CD4+ T cells (Fig. 4A), demonstrating the inhibitory effect of SOCS-1 in vivo on Th1 differentiation. Next, SOCS-1+/− and SOCS-1−/− mice were infected with N. brasiliensis, a nematode which induces Th2 cell differentiation in mice. SOCS-1−/− CD4+ T cells again produced larger amounts of IL-4 than SOCS-1+/− CD4+ T cells (Fig. 4B). In addition, enhanced Th1 response after N. brasiliensis infection was also mani-
Fig. 3. Enhanced potential of SOCS-1+/± CD4+ T cells to differentiate into Th1 and Th2 cells in vitro. (A and B) CD4+ T cells of SOCS-1+/± mice (n = 3) and their littermate SOCS-1+/+ mice (n = 3) were stimulated in vitro with plate-bound anti-CD3 plus anti-CD28 in the presence of IL-2. For Th1 differentiation, IL-12 and anti-IL-4 were added to the culture media, and for Th2 differentiation, IL-4, anti-IL-12 and anti-IFN-γ were added. After 4 days, these cells were harvested and re-stimulated with plate-bound anti-CD3 plus anti-CD28 for 24 h. The cytokine concentrations in culture media were determined by ELISA. Results are shown as means ± SD. (C) Naive CD4+ T cells were stimulated as above and re-stimulated with plate-bound anti-CD3 for 5 h. Cells were stained with anti-CD4 and indicated anti-cytokine antibodies, and analyzed by flow cytometry.

Fig. 4. Excessive Th1 and Th2 responses in SOCS-1+/± mice. Mice were challenged with L. monocytogenes (LM) (A) and N. braziliensis (Nb) (B and C). Fourteen days later, splenic CD4+ T cells were stimulated in vitro with anti-CD3 for 48 h (A and B). Cytokine concentrations in supernatants were determined by ELISA (A and B). Sera were obtained at indicated times and IgE concentrations were also determined by ELISA (C). Results are shown as means ± SD.
Fig. 5. Sustained STAT4 activation in SOCS-1+/− T cells in response to IL-12. SOCS-1+/− and SOCS-1−/− thymocytes preactivated with concanavalin A for 3 days were stimulated with IL-4 or IL-12 for 20 min and washed vigorously. Cells were harvested at indicated times after stimulation. STAT6 and STAT4 activation was determined by Western blot using an antibody specific for phospho-STAT6 (pSTAT6) and phospho-STAT4 (pSTAT4).

fested by the significant elevation of serum IgE levels in SOCS-1−/− mice (Fig. 4C). These results indicate that Th2 differentiation is also negatively regulated in vivo by SOCS-1.

SOCS-1 deficiency leads to sustained STAT4 activation in response to IL-12

As has been reported previously, SOCS-1, at least in vitro, is capable of inhibiting a wide range of cytokines by suppressing JAK (4–6). To elucidate the molecular basis for the enhanced T1/T2 differentiation in SOCS-1−/− and SOCS-1+/− mice, the duration of JAK/STAT activation in response to cytokines was examined using thymocytes from SOCS-1−/− and SOCS-1+/− mice. Consistent with the previous observation (9), IL-4-induced STAT6 phosphorylation was significantly sustained in SOCS-1−/− T cells (Fig. 5). In addition, IL-12-induced phosphorylation of STAT4 was also sustained in SOCS-1−/− T cells (Fig. 5). Thus, enhanced T1/T2 responses of SOCS-1+/− mice can be ascribed, at least in part, to sustained signaling of cytokines such as IL-12 and IL-4.

Discussion

In this study, we first showed that lymphocytes from SOCS-1/ STAT1 and SOCS-1/STAT6 DKO mice are both partially but significantly rescued from the disease caused by SOCS-1 deficiency. This result suggests that signaling pathways of both IFN/STAT1 and IL-4/STAT6 affect the homeostasis of lymphocytes in SOCS-1−/− mice. In addition, these cytokines appear to be produced by CD4+ T cells, since splenic CD4+ T cells from SOCS-1−/− mice rapidly secreted significant amounts of IFN-γ and IL-4 in response to anti-CD3 in vitro. Collectively, SOCS-1−/− CD4+ T cells, activated spontaneously by unknown stimuli in vivo, may differentiate not only into T1 cells but also into T2 cells.

Enhanced T1/T2 responses were also observed in SOCS-1+/− CD4+ T cells. Interestingly, although freshly isolated SOCS-1+/− CD4+ T cells showed normal expression of CD62L and CD69 (data not shown) and normal production of IL-2 in vitro (Fig. 2A), these cells rapidly acquired the ability to produce IFN-γ and IL-4 in response to anti-CD3 and anti-CD28 stimulation. SOCS-1+/− CD4+ T cells also produced larger amounts of IFN-γ as well as IL-4 than SOCS-1−/− cells on T1 and T2 culture conditions respectively. In addition, SOCS-1−/− mice challenged with L. monocytogenes and N. braziliensis in vivo exhibited enhanced T1 and T2 responses respectively. Thus, deletion of one SOCS-1 allele has some gene dosage effect and complete function of SOCS-1 is required for the proper regulation not only of T1 response, but also of T2 response. These findings are also supported by recent analyses. The gene dosage effect of SOCS1 was previously demonstrated through the analysis of SOCS-1+/−/ PRLR+/− mice (16). Furthermore, the role of SOCS-1 in T2 regulation was suggested by the finding in in vitro cultures of various SOCS-1−/− organs with enhanced production of IL-5 (17). Thus, SOCS-1 regulates the activation and differentiation of CD4+ T cells rather than the balance of T1/T2 responses.

The mice used by us and others were originally generated on a mixed genetic background of C57/BL6 and 129/Sv (7,8,10), and the mice used here have been backcrossed further on a C57/BL6 background (9). As is well known, these mice preferentially generate T1 responses rather than T2 responses. This may augment the production and toxicity of IFN-γ in SOCS-1−/− mice. As shown here, however, SOCS-1 regulates not only T1 response, but also T2 response. It is therefore of interest to cross SOCS-1−/− mice on an experimental background such as BALB/c and examine T1 regulation in the absence of SOCS-1. Such mice may exhibit a T2-dominant allergic disease which is different from that on a C57/BL6 and/or 129 background. This project is currently underway in our laboratory.

The hyper-responsiveness of SOCS-1−/− and SOCS-1+/− mice to IL-12-induced stimuli appears to be ascribed, at least in part, to the defect of these mice in the termination of several cytokine signaling. Consistent with our previous finding (8), we found that IL-4-induced STAT6 activation was prolonged in SOCS-1−/− T cells. This result suggests that the effect of IL-4, the most critical cytokine in T2 responses, is enhanced in the absence of SOCS-1. On the other hand, we and others have shown previously that IFN-γ-induced STAT1 activation is sustained in SOCS-1−/− cells (9,18). In addition, we showed here that IL-12-induced STAT4 activation was also prolonged in SOCS-1−/− T cells. These findings suggest that the effects of IL-12, the most critical cytokine in T1 responses, as well as those of IFN-γ, a cytokine that is also involved in T1 responses, are enhanced in the absence of SOCS-1. Thus, SOCS-1 insufficiency may lower the threshold for cytokine-induced activation/differentiation of CD4+ T cells, may lead to enhanced response to endogenous as well as exogenous cytokines and may finally result in enhanced effector functions of CD4+ T cells. The broad inhibitory action of SOCS-1 on cytokines including IL-4 and IL-12 is also consistent with the results of a recent analysis of transgenic mice that over-express the dominant-negative form of SOCS-1 (19). In this context, we cannot rule out the possibility that cytokines other
than IL-4, IFN-γ and IL-12 may also be involved in the enhanced responses of SOCS-1-/- T cells.

While previous analyses of T<sub>H1</sub>/T<sub>H2</sub> regulation focused mainly on the molecules that promote T<sub>H1</sub> differentiation, several molecules that negatively regulate T<sub>H1</sub> differentiation were also reported. These include Bcl-6 (20), NFATp(NFAT1)/NFAT4 (21), CTLA-4 (22) and DR6 (23). Interestingly, these molecules appear to limit T<sub>H2</sub> differentiation and mice lacking these factors except for DR6 exhibit T<sub>H2</sub>-dominant fatal lymphoproliferative diseases. It is of note that mice lacking SOCS-1, as reported repeatedly, exhibit a fatal disease with the infiltration of mononuclear cells in several organs. However, SOCS-1 differs from the negative regulators described above, since it appears to inhibit both T<sub>H1</sub> and T<sub>H2</sub> differentiation. In either case, these results show that the process of T<sub>H</sub> differentiation must be critically regulated at multiple steps to prevent fatal organ injuries due to over-activation of effector T cells.

Since complete lack of SOCS-1 resulted in early lethality in mice, it appears unlikely that genetic alteration of SOCS-1 which completely eliminates its action leads to some hereditary disease in humans. However, previous studies have revealed the involvement of SOCS-1 functions in human diseases. These studies demonstrated that insufficient induction of SOCS-1 mRNA due to the methylation of CpG islands of SOCS-1 promoter correlates with the occurrence and/or progression of hepatocellular carcinoma (HCC) (24,25). Because the lack of one SOCS-1 allele is sufficient to cause hyper-responsiveness to T<sub>H1</sub>-inducing stimuli, reduced induction of SOCS-1 in lymphocytes as in HCC cells or genetic mutation resulting in a partial loss of SOCS-1 function and/or induction may enhance the responses to pathogens, which may result in their rapid clearance or, in some cases, enhanced tissue injuries by hyper-activated immune systems. It is also conceivable that these conditions may promote the progression of T<sub>H1</sub>/T<sub>H2</sub>-related diseases such as multiple sclerosis and bronchial asthma. Thus, SOCS-1 is a candidate gene that influences the individual susceptibility to infections and several immune disorders. Future studies are required to fully elucidate the relevance of SOCS-1 to human diseases.

Acknowledgements
We thank Dr R. D. Schreiber and Dr S. Akira for the provision of STAT1-/ and STAT6-/ mice respectively. We also thank Mr Matsumoto and Mr Tani for their management of mice, and Ms A. Saito and Ms M. Simbo for their secretarial assistance. This study was supported by a Grant-in-Aid and Hitec Research Center Grant from the Ministry of Education, Science and Culture, Japan.

Abbreviations

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<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>DKO</td>
<td>double knockout</td>
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<tr>
<td>HCC</td>
<td>hepatocellular carcinoma</td>
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<td>SOCS</td>
<td>suppressor of cytokine signaling</td>
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References


